Transcatheter implantation of homologous “off-the-shelf” tissue engineered heart valves with self-repair capacity: long term functionality and rapid in vivo remodeling in sheep

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Abstract: Objectives: To evaluate long-term in-vivo functionality, host cell repopulation and remodeling of “off-theshelf” tissue-engineered transcatheter homologous heart-valves. Background: Transcatheter valve implantation has emerged as minimally-invasive alternative to conventional surgery in particular in elderly high-risk patients. However, currently used bio-prosthetic transcatheter valves are prone to progressive dysfunctional degeneration limiting their use in younger patients. To overcome these limitations the concept of tissue-engineered heart-valves with self-repair capacity has been introduced as next generation technology. Methods: In-vivo functionality, host cell repopulation, and matrix remodeling of tissue engineered homologous transcatheter heart valves (TEHVs) was evaluated up to 24weeks as pulmonary-valve replacements (transapical access) in sheep (n=12). As a control, tissue-composition and -structure were analyzed in identical not implanted TEHVs (n=5). Results: Transcatheter implantation was successful in all animals. Valve functionality was excellent displaying sufficient leaflet motion and coaptation with only minor paravalvular leakage in some animals. Mild central regurgitation was detected after 8 weeks increasing to moderate after 24weeks, correlating to a compromised leaflet coaptation. Mean and peak transvalvular pressure-gradients were 4.4±1.6 and 9.7±3.0mmHg. Significant matrix-remodeling was observed in the entire valve and corresponded with the rate of host cell repopulation. Conclusion: For the first time, the feasibility and long-term functionality of transcatheter based homologous off-the-shelf tissue-engineered heart-valves are demonstrated in a relevant preclinical-model. Such engineered heart-valves may represent an interesting alternative to current prostheses because of their rapid cellular repopulation, tissue remodeling and therewith self-repair capacity. The concept of homologous off-the-shelf tissue engineered heart-valves may therefore substantially simplify previous tissue-engineering concepts towards clinical translation.

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Transcatheter implantation of homologous "off-the-shelf" tissue engineered heart valves with self-repair capacity: *long term functionality and rapid in vivo remodeling in sheep*

Short title: Tissue-engineered heart valves with self-repair capacity

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None

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Abstract (250 words)

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Methods: In-vivo functionality, host cell repopulation, and matrix remodeling of tissue engineered homologous transcatheter heart valves (TEHVs) was evaluated up to 24weeks as pulmonary-valve replacements (transapical access) in sheep (n=12). As a control, tissue-composition and -structure were analyzed in identical not implanted TEHVs (n=5).

Results: Transcatheter implantation was successful in all animals. Valve functionality was excellent displaying sufficient leaflet motion and coaptation with only minor paravalvular leakage in some animals. Mild central regurgitation was detected after 8 weeks increasing to moderate after 24weeks, correlating to a compromised leaflet coaptation. Mean and peak transvalvular pressure-gradients were 4.4±1.6 and 9.7±3.0mmHg. Significant matrix-remodeling was observed in the entire valve and corresponded with the rate of host cell repopulation.

Conclusion: For the first time, the feasibility and long-term functionality of transcatheter based homologous off-the-shelf tissue-engineered heart-valves are demonstrated in a relevant preclinical-model. Such engineered heart-valves may represent an interesting alternative to current prostheses because of their rapid cellular repopulation, tissue remodeling and therewith self-repair capacity. The concept of homologous off-the-shelf tissue engineered heart-valves may therefore substantially simplify previous tissue-engineering concepts towards clinical translation.

Introduction

Valvular heart disease is an important cause of morbidity and mortality [1]. The number of patients requiring heart valve replacements is approximately 280.000 annually worldwide [2] and is estimated to triple over the upcoming decades [3]. In recent years, transcatheter valve implantations have emerged as minimally invasive alternative to conventional surgery, in particular for elderly high-risk patients. However, being bioprosthetic in nature (consisting mainly of fixed, ovine or porcine tissues) the currently used transcatheter prostheses are inherently associated with progressive dysfunctional degeneration preventing their broader use in younger patient populations.

While preliminary attempts with decellularized xenogeneic and allogeneic heart valves have only shown limited host cell repopulation in preclinical and clinical trials [4-8], the concept of tissue engineered, living and autologous heart valves with self-repair and remodelling capacity has been proposed as a promising alternative to overcome such limitations [9-15]. In 2010, we reported successfully merging transcatheter based technologies with heart valve tissue engineering based on stem cell methodology [12]. However, this “classical” heart valve tissue engineering concept comprising complex multi-step procedures such as cell harvest, cell expansion, seeding on scaffolds, bioreactor in vitro culture and time-critical implantation coordination of the delicate, living engineered autologous heart valves requires high logistical and financial efforts. In this regard, the concept of off-the-shelf (decellularized) homologous tissue engineered heart valves has been recently introduced as a promising alternative to substantially simplify current heart valve tissue engineering approaches towards routine clinical translation [16-18].

In this study and for the first time, we investigate the long-term in-vivo functionality, host repopulation capacity, and matrix remodeling of “off-the-shelf” homologous transcatheter tissue engineered heart valves (TEHVs) as pulmonary valve replacement in sheep.

Materials and methods

In vitro production of homologous cell based tissue engineered transcatheter heart valves (TEHVs): Living stented TEHV were engineered as previously described [16,19] based on rapid degrading synthetic scaffolds and vascular-derived cells and decellularized using a series of enzymatic treatments [16]. Please see supplementary file 1 for further details. Sterilization was obtained by immersion in 70% EtOH and antibiotic treatment. Decellularized TEHV were stored in fresh M-199 medium at 4^{o}C.

Transapical implantation and in-vivo performance of TEHVs: To enable transapical delivery, the stented TEHVs (length=27mm, outer diameter=30mm) were crimped and loaded onto a custom-made inducing system (OD=12mm), as described previously [12]. To evaluate in-vivo functionality and host repopulation capacity, TEHVs (n=12) were trans-apically implanted as pulmonary valve replacement in adult sheep (pulmonary annulus 24-26mm, age of 2.8±0.1 years; weight range of 69±2 kg). The remaining valves (n=5) served as reference (control) valves. All animals received humane care. The ethics committee (Veterinäramt, Gesundheitsdirektion, Kanton Zürich [197/2010]) approved the study in compliance with the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH publication No. 85-23). The implantation was carried out as previously described [12]. Please see supplementary file 1 for further details. The appropriate position and functionality of the implanted valve was visualized by angiography. In-vivo functionality (heart rate, mean and maximum trans-valvular pressure gradient, and grade of insufficiency) was monitored using trans-thoracic echocardiography during the procedure, immediately after implantation and after 1, 4, 8, 16 and 24 weeks postoperatively. Insufficiency (central regurgitation) was graded as none to trivial (1), mild (2), moderate (3), and severe (4). Anticoagulation therapy was maintained for 7 days after implantation. The animals were sacrificed and valves were explanted within 1 day (n=2), 8 weeks (n=2), 16 (n=4), and 24 weeks (n=4). Photographs were taken from all explanted valves to macroscopically analyze tissue appearance.

Qualitative tissue analyses – (immuno-) histology and SEM: Control (n=5) and explanted TEHVs (n=12) were analyzed by (immuno-) histology and scanning electron microscopy (SEM). Please see supplementary file 1 for further details.

Quantitative tissue analyses – tissue composition: The total amount of DNA, sulphated glycosaminoglycans (sGAGs) and collagen in the leaflets of control (n=5) and explanted TEHVs after 8 (n=2), 16 (n=4), and 24 weeks (n=4) as well as of native ovine valve leaflets (n=3) were analyzed using biochemical assays. For each valve 2 samples per leaflet were analyzed, resulting in 6 measurements per valve. These measurements were averaged to represent an average value for each valve. The values for DNA, sGAGs and collagen are expressed per mg dry weight. Please see supplementary file 1 for further details on the assays.

Statistics: Data are represented as average values ± standard-deviation. To identify differences in tissue composition (DNA, sGAG and collagen) between the control valve leaflets and the explanted leaflets at 16 and 24 weeks and that of native ovine valve leaflets one-way ANOVA was performed with Tukey's post-hoc testing. The number of values for the explanted valves after 8 weeks was too low (n=2) to include in the statistical analyses. Differences were considered significant when $p<0.05$. Statistics were performed using GraphPad Prism software (version 5.0d, San Diego, USA).

Results

Implantation and in-vivo performance of TEHV's: The transapical implantation procedures (n=12) were successful. No perioperative morbidity or mortality occurred and all valves could be deployed successfully at the target-site (Fig. 1; Suppl. Video 1). In the early postoperative phase, two animals presented with valve migration into the right-ventricular outflow-tract and died within 24 hours postoperatively. The remaining animals (n=10) made a swift recovery and completed their respective follow-up without any complications. Serial echocardiography confirmed sufficient valve function with mobile leaflets and excellent coaptation at the early follow-up time-points (Fig. 2; Suppl. Videos 2 and 3). None central regurgitation was observed at 4 weeks (n=10) and mild central regurgitation at 8 weeks (n=10) (table 1). Only minor paravalvular leakage was initially observed in some animals up to one week after implantation.

Long-term follow-up: While leaflet mobility was maintained on long-term follow-up, the coaptation slowly decreased over time (Fig. 2, Suppl. Video 4), which was most likely due to merging of the leaflets with the valvular wall at the level of the hinge area as well as the occurrence of a single leaflet prolapse in some animals. Consequently, mild to moderate central regurgitation could be observed at 16 weeks (n=8), that further increased to moderate central regurgitation at 24 weeks (n=4) with one animal presenting with severe insufficiency. Functional measurements (table 1) demonstrated stable mean and peak transvalvular pressure gradients over time (4.4±1.6 and 9.7±3.0 mm Hg respectively).

Macroscopic TEHV appearance: The implanted in-vitro grown homologous TEHV revealed thin and shiny tissue formation in both valvular wall and leaflets (Fig. 3A-C). All explanted TEHVs demonstrated shiny and pliable leaflets and dense whitish valvular wall tissue, irrespective of the implantation period (Fig. 3D-L). In all explants, the valvular wall tissue was integrated into the surrounding native valvular wall. Excellent coaptation of the explanted valves was evident in the implant (Fig. 3A) and was maintained up to 8 weeks of implantation (Fig. 3D). Thereafter, valve closure was incomplete in line with the observed central regurgitation (Fig. 3G,J). Apparently, the line of attachment of the leaflets to the valvular wall shifted upwards in time indicating tissue merging process at the level of the hinge area (Fig. 3E,F,H,I,K,L), associated with a reduction in leaflet size with time.

TEHV repopulation and remodeling: Prior to implantation the TEHVs revealed no cellular remnants (Fig. 4A,F,K) and a well-developed extracellular matrix, mainly consisting of collagen demonstrating an efficient decellularization procedure (Fig. 4P,U,Z). After TEHV deployment in the pulmonary positions endogenous cellular repopulation occurred rapidly with first signs of cell infiltration as early as 5 hours after implantation (Fig. 4B,G,L). Both leaflets and wall tissues were homogeneously repopulated over time (Fig. 4C,D,H,I,M,N) with fastest repopulation at highest densities in the wall. After 24 weeks, cell repopulation density in the leaflets (Fig. 4E) and hinge area (Fig. 4J) approached that in the valvular wall (Fig. 4O). Scaffold remnants remained longest visible in the leaflets (Fig. 4B,C,D,E) with local increased cell densities. Minimal depositions, most likely blood platelets and fibrin, were present at the surface of the whole valve after 5 hours (Fig. 4B,G,L), but disappeared with time. The valvular tissues demonstrated abundant amounts of collagen that increased in density over time, in particular in the hinge area (Fig. 4V,W,X,Y) and wall (Fig. 4AA,BB,CC,DD) and to a lesser extent in the leaflets (Fig. 4Q,R,S,T). Elastic matrix formation was evident in the wall at 8 weeks and later time points (Fig. 4BB,CC,DD). In the hinge area the formation of elastic fibers was visible after 16 weeks (Fig. 4X,Y) and in the leaflets at 24 weeks (Fig. 4T). Calcification was not detected in any of the valves (data not shown).

<u>TEHV cell phenotypes and distributions:</u> Cells infiltrating the valve within 5 hours after implantation were all vimentin positive (Fig. 5A,E,I) and α-SMA negative (Fig. 5M,Q,U). After 8 weeks, vimentin-positive cells were identified mainly in close vicinity of the polymeric scaffold remnants in the leaflet (Fig. 5B) and hinge area (Fig. 5F), while more homogenously distributed and in higher amounts in the valvular wall (Fig. 5J). The cells in the leaflet and hinge area were all α-SMA negative (Fig. 5N,R) and α-SMA positive in the valvular wall (Fig. 5V). After 16 weeks, homogenously distributed vimentin-positive cells were observed in all regions of the valve (Fig. 5C,G,K). These cells were α-SMA positive in the hinge area (Fig. 5S) and wall (Fig. 5W) and sparsely α-SMA positive in the leaflet (Fig. 5Q). After 24 weeks, vimentin-positive cells were homogenously distributed over the valve (Fig. 5D,H,L). The level of α-SMA seemed lower in the hinge area (Fig. 5T) and wall (Fig. 5X) as compared to the 16 weeks explants. In the leaflets, more α-SMA positive cells were identified (Fig. 5P) as compared to earlier time points.

After 8 weeks TEHV demonstrated partly confluent endothelial lining as observed by CD31 staining (Fig. 6A,D,G). Similar features were observed at the surface of the explants at 16 (Fig. 6B,E,H) and 24 weeks (Fig 6C,F,I). The cell lining showed the typical cobblestone morphology at all time points, representative for endothelial cells as visualized by SEM (Fig 6J-O). The degree of endothelialization varied between locations and explants with decreasing endothelialization at the hinge area with implantation time (Fig. 6D,E,F) and increasing endothelialization of the leaflet (Fig. 6A,B,C) and valvular wall surface with implantation time (Fig. 6G,H,I).

Quantitative TEHV tissue analyses: DNA content (Fig. 7A) of the leaflets increased with implantation time as compared to the values before implantation ($p<0.01$ at 16 weeks and $p<0.001$ after 24 weeks). After 16 weeks, DNA content was still lower than that in native leaflets ($p<0.05$), but after 24 weeks the DNA content in the explanted TEHVs was similar to that in native ovine valve leaflets. The amount of sGAGs (Fig. 7B) was lower in TEHVs before implantation as compared to that in native leaflets ($p<0.01$) and was still lower in the 16-week explants ($p<0.05$). After 24 weeks sGAG content approached native values. Collagen content (Fig. 7C) was higher after 16 and 24 weeks in-vivo as compared to the values of the TEHVs before implantation ($p<0.05$). Collagen content was equal to that in native ovine leaflets at all time points.

Discussion

Since the emergence of transcatheter heart valve implantations, which today are mostly used in elderly inoperable patients, an expansion to younger patient populations is anticipated in the near future. A mayor limitation however -as in classical surgical bioprostheses- is the progressive dysfunctional deterioration inherent to the non-living animal derived tissue used in today's bioprostheses. Furthermore, accumulating clinical findings suggest that such degenerative processes may be even aggravated in trans-catheter valves due to the substantial mechanical stresses during the crimping and deployment procedure. In the search for a next generation technology platform to overcome the limitations of today's bioprosthetic transcatheter heart valve prostheses, the concept of heart valve tissue engineering (HVTE) using living autologous tissues with self-repair capacity has created substantial hope for future heart valve therapy concepts. In 2010, we have demonstrated the principal feasibility to merge transcatheter based technologies and HVTE demonstrating transcatheter implantation of stent based tissue engineered heart valves as pulmonary valve replacements in a preclinical animal model [12]. However, in this proof-of-concept study, the generation of the used TEHV followed the algorithm of the classical heart valve tissue engineering approach comprising complex logistical and financial efforts including cell and expansion, scaffold seeding, bioreactor in vitro preconditioning and time-critical implantation coordination of the delicate, living, autologous tissue engineered heart valves.

In this study we simplified the previously used HVTE concept towards a substantially more translational and clinically relevant methodology demonstrating long-term in-vivo functionality, host repopulation capacity, and matrix remodeling of off-the-shelf, tissue-engineered decellularized homologous transcatheter TEHV in sheep. Conceptually, off-the-shelf, homologous tissue engineered heart valves carry significant advantages either when compared to decellularized xenogeneic/allogeneic natural heart valves [16,18] or to the technologically and logistically demanding "classical" heart valve tissue engineering (HVTE) approaches. By decellularization of homologous TEHV based on biodegradable PGA/P4HB scaffolds, we are able to produce largely available off-the-shelf homologous starter matrices at any clinically relevant size without the risk for xenogeneic disease transmission [16]. Importantly, this off-the-shelf concept greatly simplifies previous HVTE concepts with regards to financial and logistical efforts

[16]. Last but not least, these decellularized matrices are designed and adapted for trans-catheter implantation, therewith further enhancing their clinical relevance.

Overall the TEHV function was sufficient with mobile leaflets and excellent coaptation at the early follow-up time-points. No central regurgitation was observed at 4 weeks and mainly mild central regurgitation at 8 weeks. While leaflet mobility as well as a low transvalvular mean gradient was maintained on all follow-up time points, valvular coaptation slowly decreased over time, which was most likely due to a principal design flaw of these prototype TEHVs resulting in merging of the leaflet base with the respective hinge areas. This may be primarily related to the prototype stent- and valve design lacking important anatomical features such as sinuses and thus leading to non-physiological loading and insufficient washout in the hinge area in particular during diastole.

To ensure viability, prevent deterioration, and allow for growth and remodeling it is crucial that the host rapidly repopulates these matrices after implantation. In our study, the TEHVs demonstrated a remarkable repopulation and remodeling capacity, pointing to the high potential of this approach with regard to self-repair capacity.

Repopulation occurred rapidly already after a few hours. It was fastest in the valvular wall, followed by the hinge area and the leaflets, reaching similar DNA content to that of native valves. In line with that, the collagen amount reached native levels with increasing density over time and the formation of elastic fibers was visible. Remodeling of the matrix occurred throughout the whole valve, but fastest in the wall, followed by the hinge area and the leaflets and as such correlates with the observed rates of repopulation. Furthermore, endothelialization of the surfaces occurred at the valvular wall and the leaflets.

In comparison to native decellularized allogenic or xenogenic valve prostheses that have been repeatedly reported to show if at all only limited cell repopulation [4-8], repopulation of our TEHVs appears to be substantially more efficient. Importantly, thus far, repopulation of valve starter matrices was assumed to occur from the valve basis upward associated with remodeling of the valve matrix. This study suggests a potentially faster and more important route of repopulation, which is by blood-borne cells. The efficacy of repopulation and subsequent production and remodeling of extracellular matrix via this route might be very potent and its mechanisms are subject to subsequent studies.

Translation of this homologous off-the-shelf heart valve tissue engineering from the low-pressure pulmonary circulation to the systemic circulation appears feasible. Recent pilot experiments by our group have demonstrated structural integrity at systemic pressures of comparable valves [20-22] As soon as the stent and valve delivery system have been adapted to the aortic root, future studies will focus on long-term systemic valve replacements.

In conclusion, we demonstrate for the first time feasibility and long-term functionality of transcatheter delivered homologous off-the-shelf tissue engineered heart-valves with the potential for self-repair. The concept of off-the-shelf TEHV may represent a promising alternative to currently used valve prostheses as of their rapid host cell repopulation and remodeling capabilities towards native valve features within a short time-span. Moreover, the off-the-shelf TEHV concept may significantly simplify previous, classical heart valve tissue-engineering concepts towards clinical translation. Further stent- and scaffold design modifications and optimizations (i.e. the implementation of the anatomical sinuses) are necessary to ensure and further improve long term functionality of these next generation valves.

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Figure legends

Figure 1: Angiography of the implantation procedure. Function of the native valve was assessed prior to implantation (A,B). The inducing system was inserted (C) and the valve was delivered into the pulmonary artery (D,E). Afterward, the position and functionality of the implanted valve was visualized (F).

Figure 2: Echocardiography of TEHV. Representative examples of valve behavior directly after implantation (A-C), after 4 weeks (D-F), 8 weeks (G-I), 16 weeks (J-L), and 24 weeks (M-O). Valve performance was excellent with mobile and coapting leaflets at early follow-up time points (A,B,D,E). Mobility of the leaflets was maintained, but coaptation decreased with over time (G,H,J,K,M,N) leading to central regurgitation after 16 (L) and 24 weeks follow-up (O).

Figure 3: Macroscopic appearance of the implanted (A-C) and explanted (D-L) decellularized in-vitro grown TEHV in closed configuration (A,D,G,J), opened configuration (B,E,H,K) and as cross-section (C,F,I,L)**.** The implants and explants all revealed shiny and pliable leaflets. Complete closure was evident in the implant (A) and was maintained up to 8 weeks (D), while being incomplete thereafter (G,J). The line of attachment of leaflets to the wall moved upwards in time (marked with arrows), associated with a reduction in leaflet size (E,F,H,I,K,L).

Figure 4: Histology (H&E in A-O and EvG in P-DD) of the implanted and explanted TEHV. The implants revealed no cellular remnants (A,F,K) and a well-developed extracellular matrix (P,U,Z). The valvular tissues were homogeneously repopulated over time (B,C,D,E,G,H,I,J,L,M,N,O) with fastest repopulation at highest densities in the wall. Scaffold remnants (indicated by solid arrows) remained longest visible in the leaflets (A-E). Minimal depositions (indicated by asterisks) were present at the surface after 5 hours (B,G,L), but disappeared with time. The valvular tissues demonstrated abundant amounts of collagen that increased in density over time (Q,R,S,T,V,W,X,Y,AA,BB,CC,DD), mostly in the hinge area and wall. Elastic matrix formation (in black, indicated by dashed arrows) was evident in all parts of the valve, appearing fastest in the wall (BB, CC, DD), followed by the hinge area (X,Y) and the leaflets (T). Scale bars represent 200µm.

Figure 5: Cellular phenotypes (vimentin in A-L and α-SMA in M-X) in explanted TEHV. Vimentin-positive cells were observed in all explants at all time points (A-L), with high cell densities near the scaffold remnants. In the leaflets, no α-SMA expression was observed after 5 hours (M) and 8 weeks (N). First signs of α-SMA-positive cells appeared at 16 weeks (O) and its level increased after 24 weeks (P). The hinge area demonstrated similar features (Q-T), though the level of expression seemed to decrease after 24 weeks (T) as compared to 16 weeks (S). The cells in the valvular wall showed no α-SMA after 5 hours (U), but abundant expression of α-SMA at 8 weeks (V) that seemed to decrease at 16 weeks (W) and further at 24 weeks (X). Scale bars represent 200μm.

Figure 6: Endothelialization of explanted TEHV as visualized by CD31 staining (A-I) and scanning electron microscopy (SEM; J-O). Endothelialization was observed as a monolayer of CD31-positive cells lining the valve explant and was confirmed by its cobblestone morphology as observed by SEM. The degree of endothelialization varied between locations and explants, but was observed at all time points. Scale bars represent 200μm (A-I) and 50μm (J-O).

Figure 7: The amount of DNA (A), sGAGs (B), and hydroxyproline (Hyp) as a measure for collagen (C) in TEHV leaflets before implantation (white bars), after 8 (light grey bars), 16, and 24 weeks in-vivo (medium grey bars), and of native ovine pulmonary valve leaflets (dark grey bars). The data for the 8-week explants was not taken into account in the statistical analyses, as the number of data points was too low (n=2). DNA content increased in time, with values comparable to that in native leaflets after 24 weeks. sGAG values are still lower than that in native leaflets after 16 weeks, but approached native values after 24 weeks. Collagen content was higher after 16 and 24 weeks as compared to the values before implantation and was similar to those in native ovine leaflets.

Supplementary Videos

Supplementary Video 1: Exemplary angiography showing transcatheter implantation of a TEHV into the pulmonary-artery (PA).

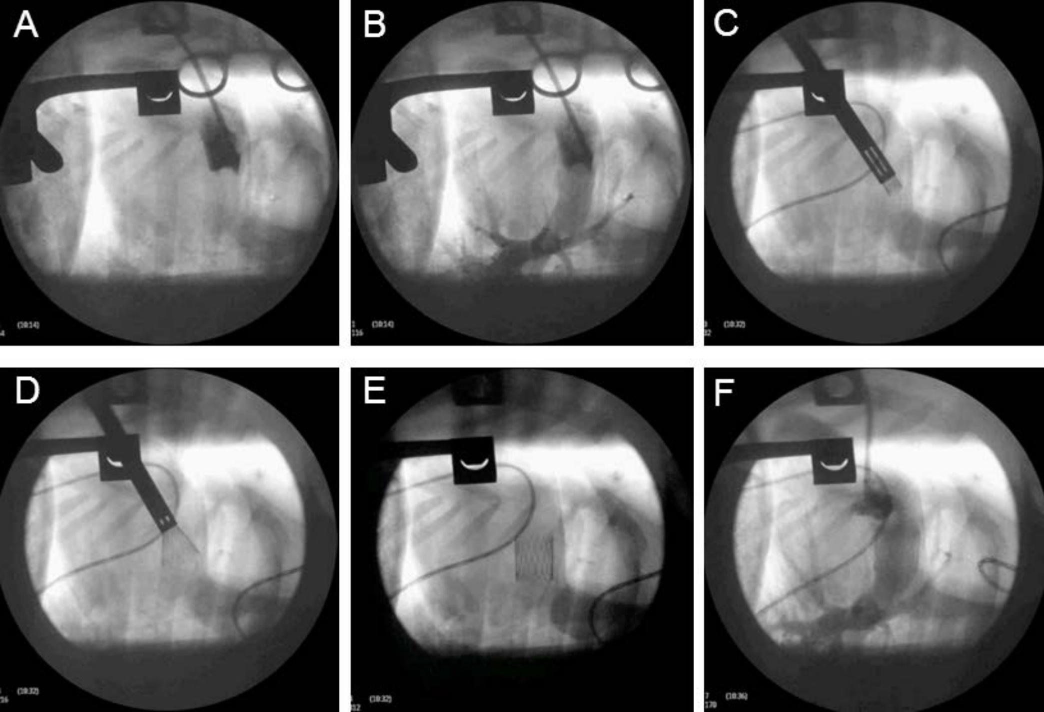
Supplementary Video 2: Exemplary echo of a TEHV in the PA at day of implantation.

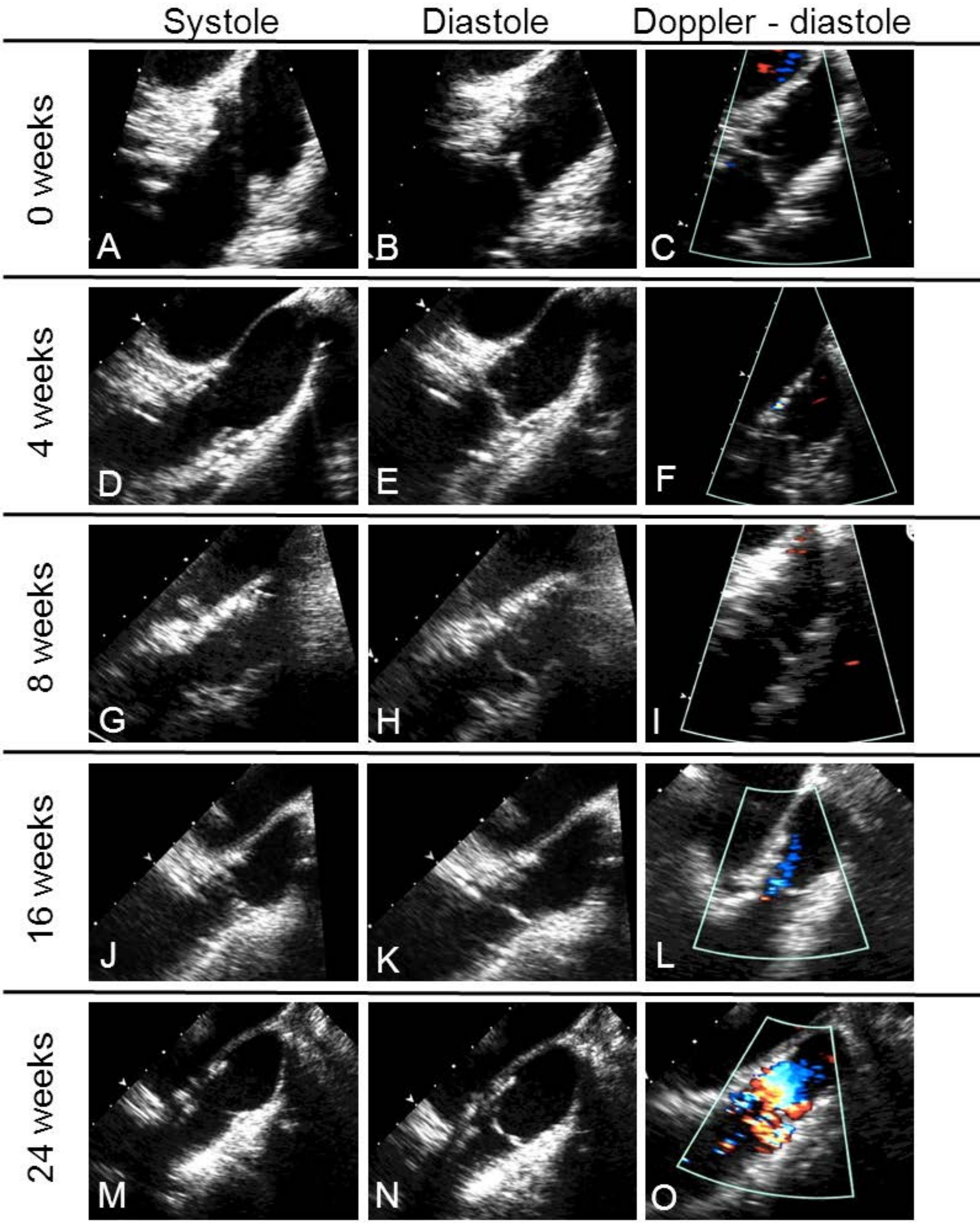
Supplementary Video 3: Exemplary echo of a TEHV in the PA at 8 weeks follow-up.

Supplementary Video 4: Exemplary echo of a TEHV in the PA at 24 weeks follow-up.

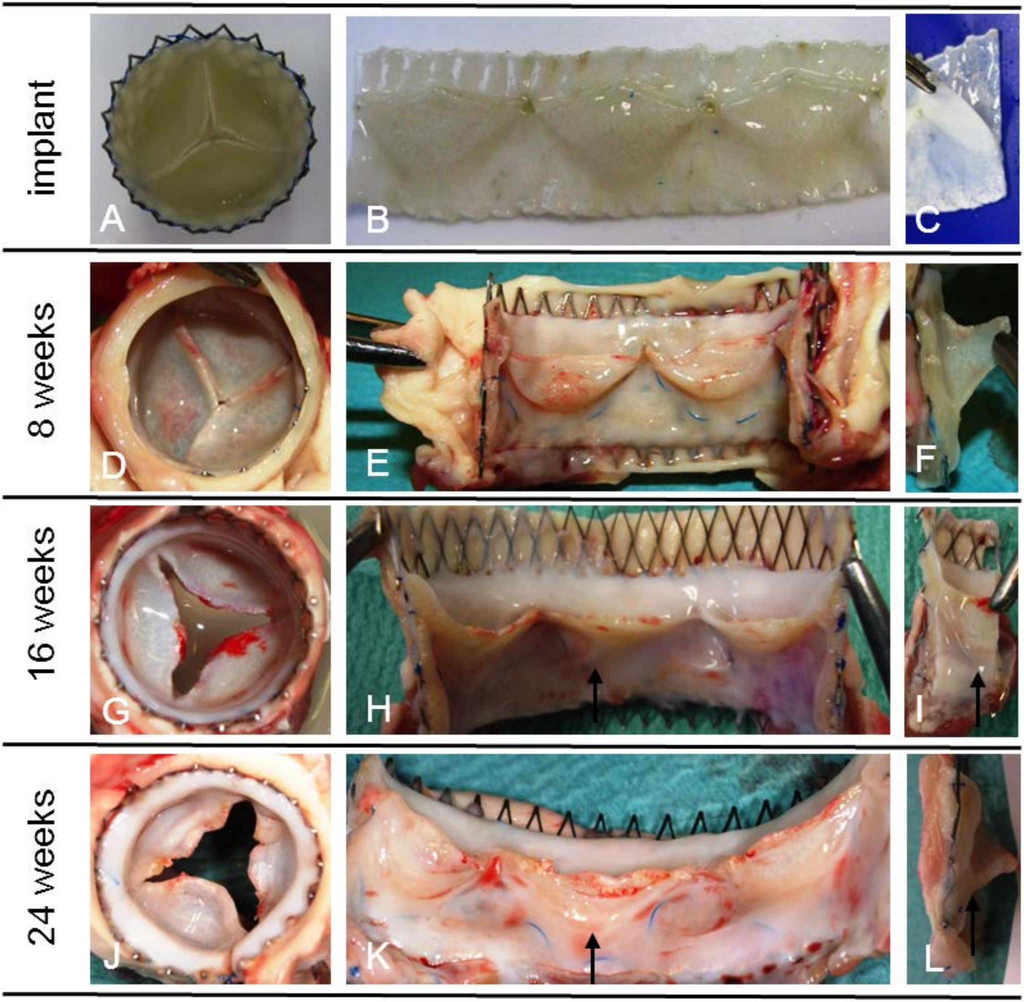
Follow-up	N	Heart rate (bpm)	Mean dP (mm Hg)	Maximum dP (mm Hg)	Insufficiency grade (mean)	Insufficiency grade (per animal)
0	11	85±18	3.5±0.7	8.2±2.7	1.2±0.4	1 *(10/11)* 2 *(1/11)*
1	10	111±17	4.5±1.0	10.4±1.8	1.2±0.4	1 *(8/10)* 2 *(2/10)*
4	10	96±18	4.3±1.1	9.2±2.3	1.1±0.3	1 *(9/10)* 2 *(1/10)*
8	10	103±19	5.1±1.3	10.8±2.9	2.0±0.9	1 *(3/10)* 2 *(5/10)* 3 *(1/10)* 4 *(1/10)*
16	8	87±21	5.0±2.8	10.3±4.8	2.6±0.9	1 *(1/8)* 2 *(2/8)* 3 *(4/8)* 4 *(1/8)*
24	4	101±4	4.2±2.0	8.7±3.6	3.2±0.5	3 *(3/4)* 4 *(1/4)*
Overall mean	-	97±20	4.4±1.6	9.7±3.0	-	-

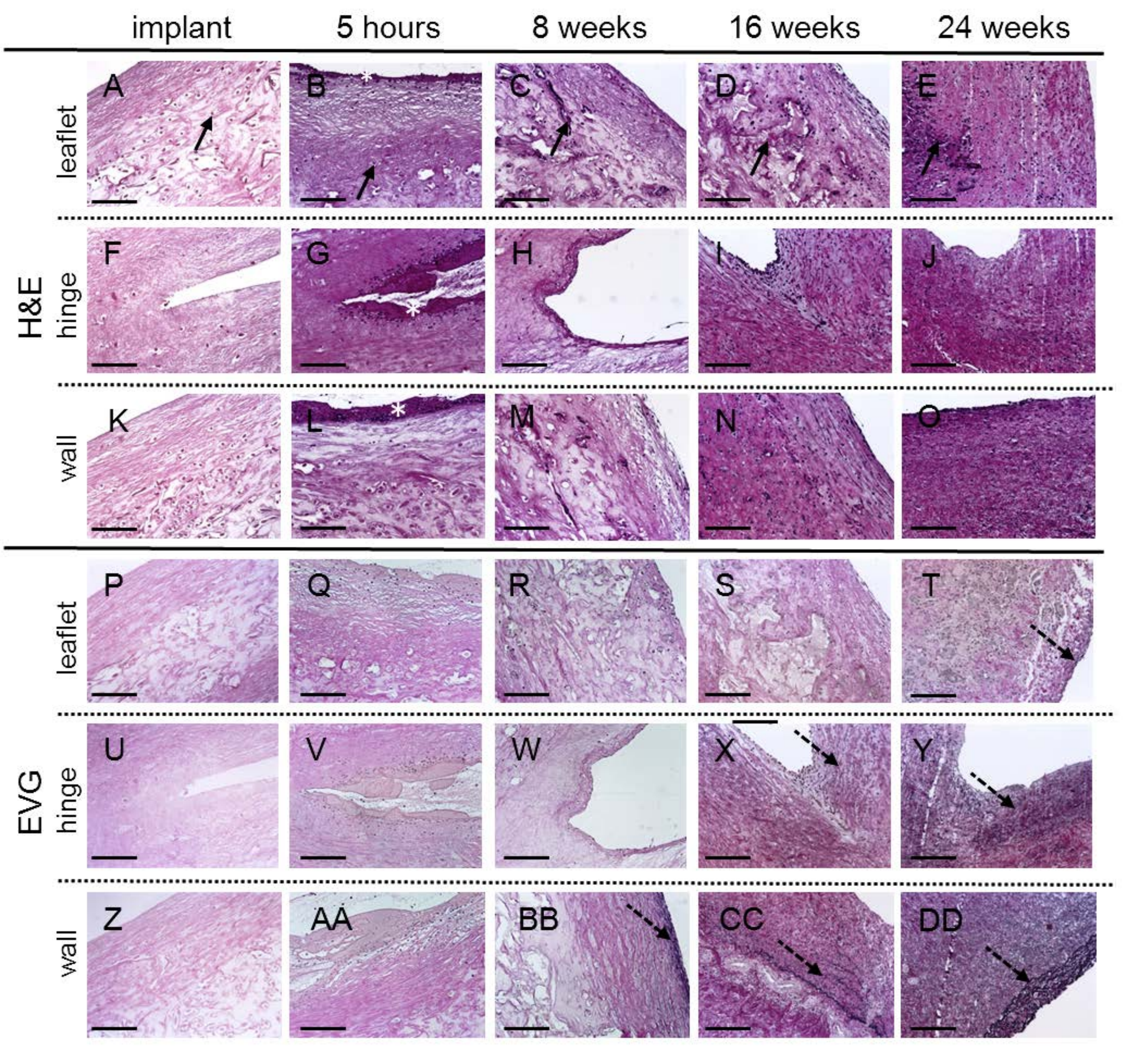
Table 1: Echocardiographic assessment of TEHV directly after implantation, at 1, 4, 8, 16, and 24 weeks follow-up. N represents the number of animals and dP represents the transvalvular pressure gradient. Insufficiency (central regurgitation) was graded as none to trivial (1), mild (2), moderate (3), and severe (4).


A
B
C
D
E
F

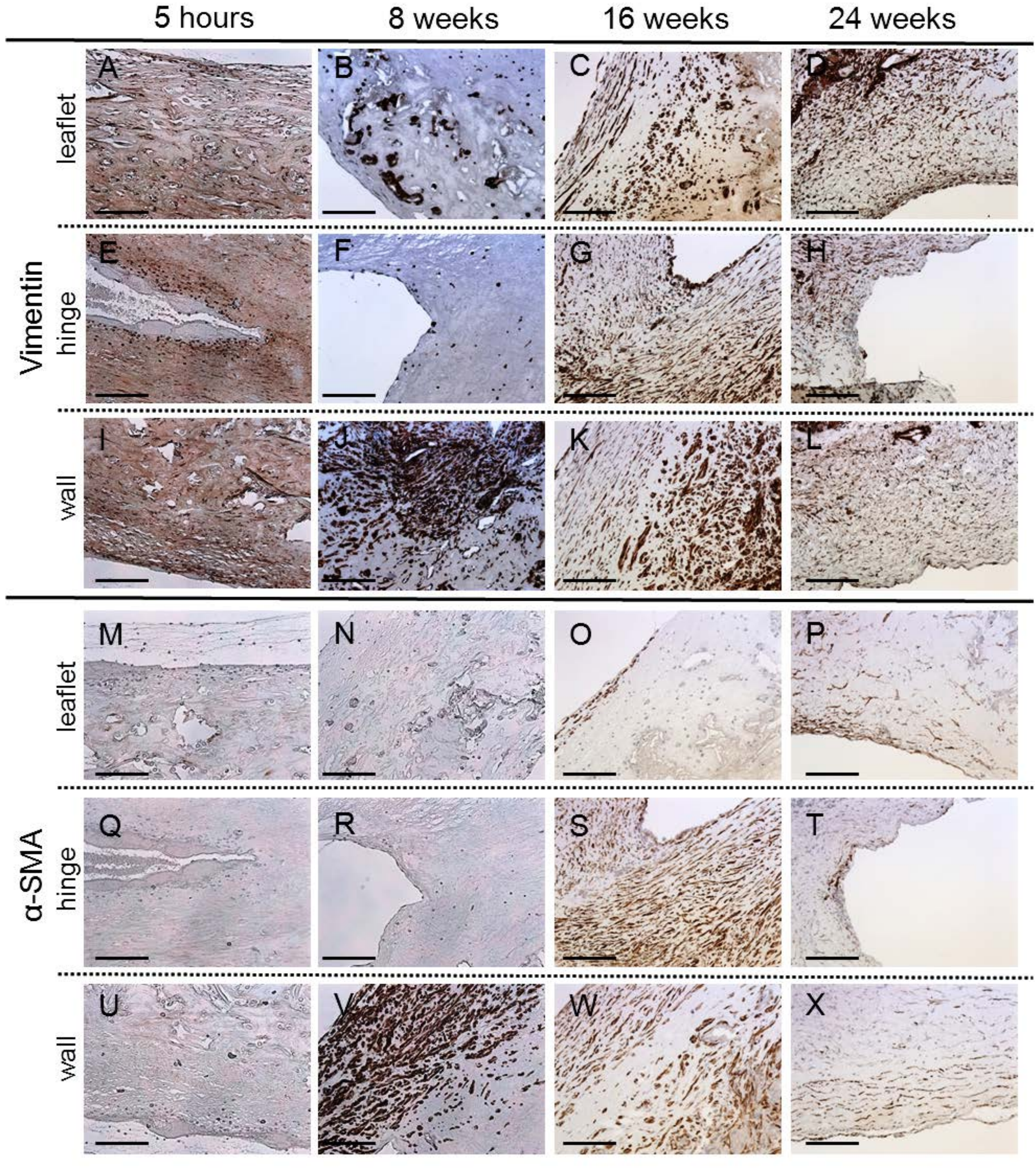


Systole
Diastole
Doppler - diastole
0 weeks
4 weeks
8 weeks
16 weeks
24 weeks
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O


implant
8 weeks
16 weeks
24 weeks
A
B
C
D
E
F
G
H
I
J
K
L



implant
5 hours
8 weeks
16 weeks
24 weeks
H&E
EVG
leaflet
hinge
wall
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
S
T
U
V
W
X
Y
Z
AA
BB
CC
DD


5 hours
8 weeks
16 weeks
24 weeks
Vimentin
α-SMA
leaflet
hinge
wall
leaflet
hinge
wall
A
B
C
D
E
F
G
H
I
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K
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N
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Q
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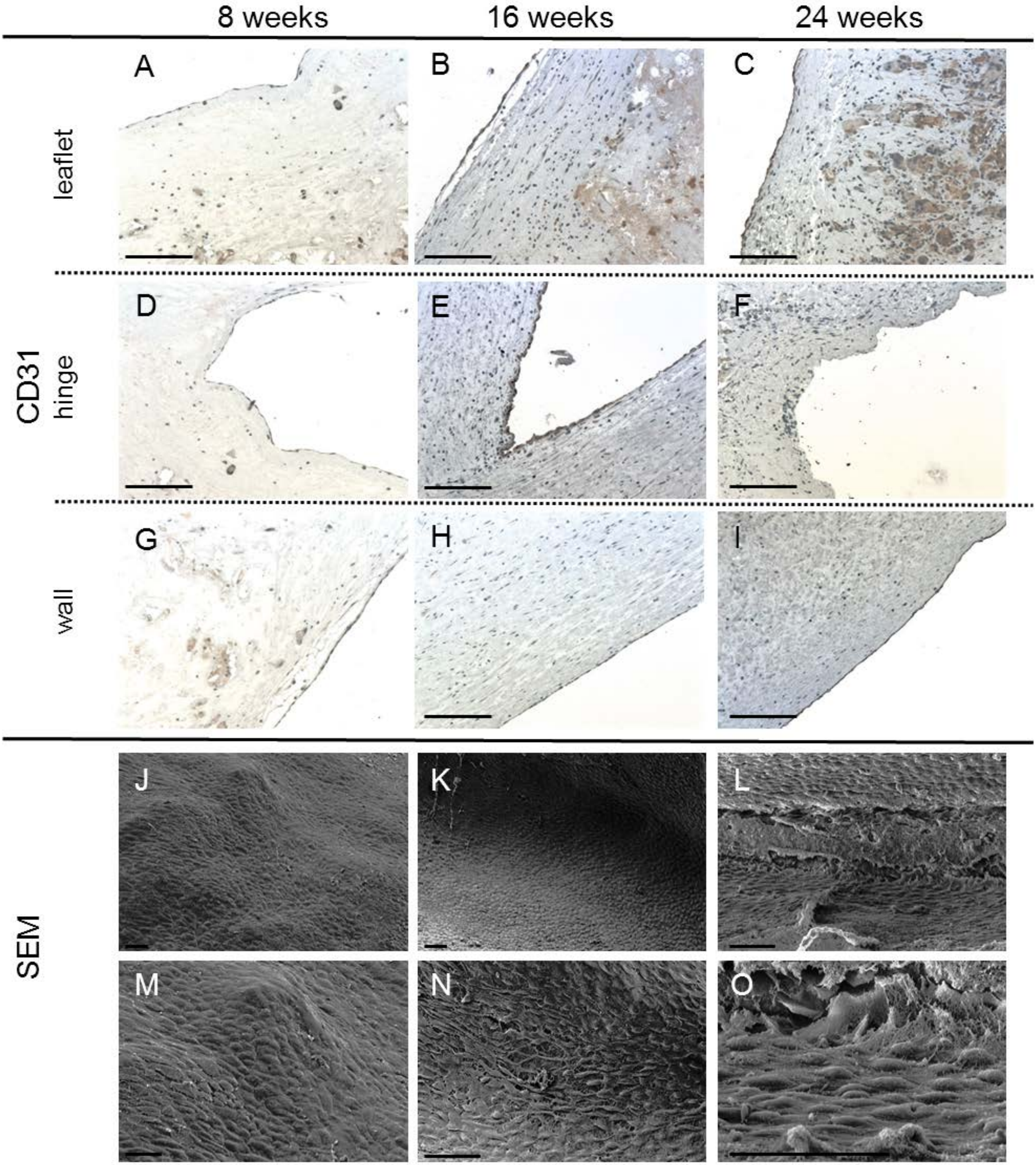
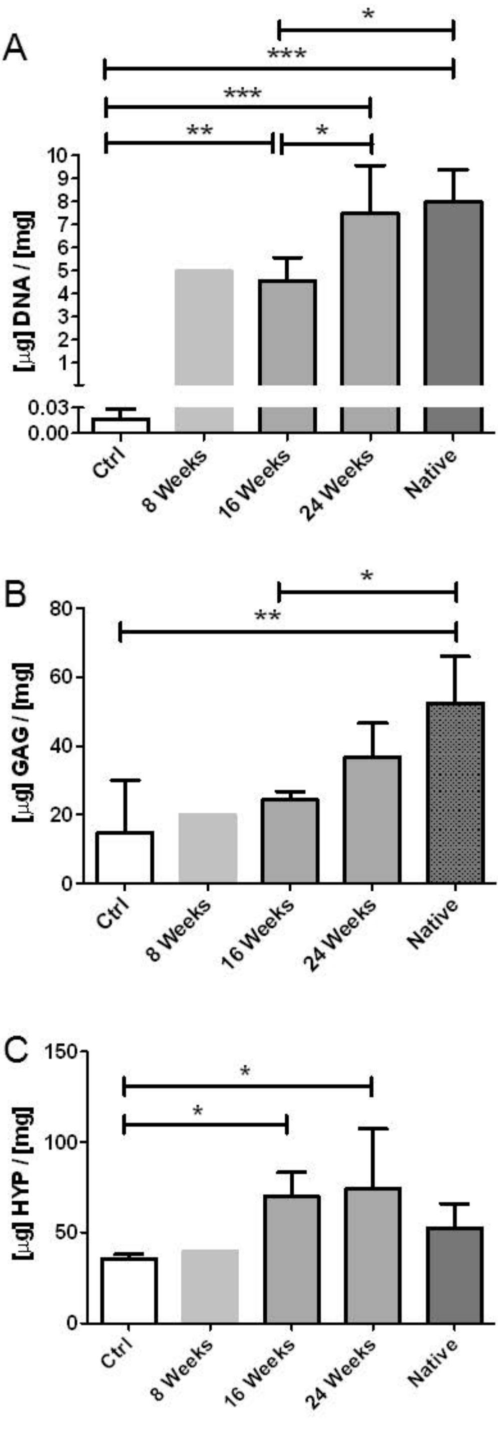

8 weeks
16 weeks
24 weeks
CD31
leaflet
hinge
wall
SEM
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O



A
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10
9
8
7
6
5
4
3
2
1
0.03
0.00
[µg] DNA / [mg]
Ctrl
8 Weeks
16 Weeks
24 Weeks
Native
B
*
**
80
60
40
20
0
[µg] GAG / [mg]
Ctrl
8 Weeks
16 Weeks
24 Weeks
Native
C
*
*
150
100
50
0
[µg] HYP / [mg]
Ctrl
8 Weeks
16 Weeks
24 Weeks
Native